Influence of previous tuberculosis treatment on time to culture conversion for patients receiving a bedaquiline-containing regimen at Sizwe Tropical Disease Hospital, South Africa

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Influence of previous tuberculosis treatment on time to culture conversion for patients receiving a bedaquiline-containing regimen at Sizwe Tropical Disease Hospital, South Africa

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Abstract

Tuberculosis (TB) remains a leading cause of death worldwide. New drug regimens were needed urgently to alleviate mortality and morbidity among drug-resistant TB patients. With the widespread use of bedaquiline (a newly developed diarylquinoline), the potential for drug-resistant strains requires optimal regimen selection and length of treatment. To limit bedaquiline resistance, information is also needed on the profile of patients who require extended duration of bedaquiline therapy. We examined whether previous exposure to TB treatment influenced the time to culture conversion (compared with no previous treatment) for patients receiving a drug-resistant (DR) TB regimen containing bedaquiline. We undertook a retrospective cohort study of DR-TB patients with documented culture conversion who had been initiated on a DR-TB regimen containing bedaquiline. The cohort was treated from April 2016 to March 2019 at Sizwe Tropical Disease Hospital. A total of 397 files were audited, 303 new and 94 previously treated DR-TB patients. We found that up to two months, the two groups experienced similar rates of culture conversion, after which point the new-patient group displayed faster times to culture conversion. The previously treated group had 4.12 times the odds of culture converting after 6 months compared to the new group. The previously treated group thus received a longer duration of bedaquiline treatment than the new-patient group ($p<0.001$). The findings of this study substantiate the potential impact of previous exposure to TB treatment and establishing the influence on the efficacy of bedaquiline therapy. Monitoring of susceptible bedaquiline MIC in previously treated patients as early as two months in the event of failure to culture convert may be warranted. In addition further evaluation of other prescribed concurrent medication which may affect bedaquiline MIC would also need to be undertaken. Such monitoring can help prevent emergent bedaquiline drug resistance.

Introduction

Tuberculosis (TB) remains one of the leading causes of death worldwide. There is a growing crisis concerning drug-resistant (DR) TB, which in 2019 accounted for approximately 500 000 incident cases. Among those cases, rifampicin-resistant (RR) or multidrug-resistant (MDR) forms of TB accounted for 78%[1]. Globally, 3.3% of new TB cases and 17.7% of previously treated cases were found to have RR or MDR TB disease [1]. Compared with drug-susceptible TB, MDR-TB has higher mortality and morbidity rates [1]. The treatment success rate for both MDR/RR-TB and extensively drug-resistant (XDR) TB is poor globally [1].

The poor success rate for DR-TB treatment is the result of high mortality, loss to follow up, adverse drug reactions and frequent side effects [1]. With use of second-line injectables in DR-TB treatment, hearing loss has been a notable finding and warrants close monitoring [2]. The treatment of MDR-TB and XDR-TB is lengthy and

expensive[1]. These are some reasons for the poor treatment outcomes. In addition, it is difficult to design an effective drug regimen using four effective drugs [3]. Hence, new drug regimens were urgently needed to reduce mortality and morbidity among DR-TB patients.

Effectiveness of bedaquiline

Bedaqualine is a newly developed diarylquinoline with a unique mechanism of action. Specifically, it inhibits mycobacterial adenosine triphosphate synthase [4]. Clinical trials have shown benefits for reduced time to sputum conversion when bedaquiline was included in a treatment regimen. Bedaquiline is now included as one of the drugs in DR-TB regimens. With the use of bedaquiline in the current RR/MDR-TB regimen, high treatment success rates (87% to 90%) have been achieved [2].

The effectiveness of therapy is based on sputum culture conversion during the initial six-month treatment period [4-6]. The results of a systematic review of the therapeutic efficacy of bedaquiline indicated that sputum conversion was evident during the first two months of treatment for 48% to 84% of patients in comparison to background regimens which included placebo among MDR-TB and XDR-TB patients [5]. The use of bedaquiline in treatment regimens for baseline culture positive pulmonary MDR-TB and XDR-TB achieved culture conversion in 79% of patients after three months and in 97% of patients after six months of treatment, respectively [6]. Reduced time to sputum culture conversion is a useful early predictor of the successful final outcome of treatment.

The World Health Organisation (WHO) has recommended that bedaquiline be added to an optimised background therapy of four effective second-line drugs; the recommendation is to use bedaquiline for up to 24 weeks (but not longer) [7]. The frequently noted adverse event identified with bedaquiline use were transaminitis, which is an increase in the liver transaminase enzymes of up to three times the upper limit of normal [7]. Of note, corrected QT prolongation on electrocardiogram, which is considered as values above 450ms or increase more than 60ms from reference values is also a frequently occurring adverse event [7].

There is limited evidence regarding the extended use of bedaquiline for MDR-TB and pre-XDR-TB patients. Much of the evidence regarding the use of bedaquiline for extended periods has been obtained from studies that have evaluated XDR-TB treatment regimens. Duration of bedaquiline use was shown to be successfully extended in XDR-TB patients with no significant adverse effects [8]. A case series report of patients with individualised XDR-TB regimens demonstrated the benefits of using bedaquiline, without adverse events related to QT interval prolongation being recorded. This was despite co-administration with clofazimine and moxifloxacin, two drugs that both have side-effect profiles of QTc interval prolongation [9]. The major concerns regarding the extended use of bedaquiline are

accumulative toxicity and the risk of QTc interval prolongation [10].

In a systematic review by Li et al., bedaquiline was shown to have favourable TB outcomes through advanced early bactericidal activity, but only when combined with other bactericidal drugs [11]. Bedaquiline should be accompanied by pyrazinamide and four second-line drugs which have been selected based on 'drug susceptibility testing (DST) and/or previous use and/or drug resistance surveillance data' [7]. Including bedaquiline in drug regimens that are suboptimal could result in selection pressure and drug-resistant mutations [12].

Several genetic targets that are associated with bedaquiline resistance have been identified. Researchers have described the presence of the Rv0678 gene mutation in evaluating bedaquiline therapy [13-15]. The Rv0678 mutation has demonstrated a more than four-fold increase for the minimum inhibitory concentrations (MIC) of bedaquiline [16]. The descriptions of Rv0678 mutation in isolates from TB patients without prior bedaquiline exposure were more pronounced in MDR-TB patients than DS-TB patients. Villelas et al. found the presence of Rv0678 mutations in patients with prior rifampicin exposure during their evaluation of mutations and the impact on treatment outcomes [15]. The significance of mutations requires further exploration, and the association between prior exposure to drugs used in first-line TB regimens and the presence of mutations warrants attention.

Previous treatment history and efficacy of bedaquiline

The drug resistance profiles of mycobacterium TB isolates influence the rate of conversion such that an inverse relationship exists between conversion rate and degree of drug resistance [17]. Previous TB treatment is regarded as an important risk factor for RR/MDR-TB, and rigorous management is required as these patients may have been infectious for a longer period [18]. Compared with new TB cases, previously treated patients showed a higher prevalence of drug resistance against first-line anti-TB drugs [19]. Studies have also shown that previous treatment history for TB is a risk factor for poor TB outcomes [20-21]. Researchers have reported varying time to culture conversion regarding RR/MDR-TB patients who have a history of previous TB treatment, despite the rapid bactericidal effect of bedaquiline [22-23].

Guglielmetti et al. evaluated the treatment effects of bedaquiline in MDR-TB and XDR-TB patients. The results showed that the time to culture conversion was adversely affected by the presence of lung cavities [6]. Relapses among patients with a previous TB history may be due to fibrotic lesions or persistent cavities [6]. A systematic review of cohort studies regarding bedaquiline indicated a risk of poor outcomes for patients who had lung cavitations and severe drug resistance (presence of resistance to Isoniazid (KatG and inhA gene mutations), Rifampicin (RpoB mutation) and/or aminoglycosides (SLID gene mutation) and /or fluroquinolones (gyrA, gyrB gene mutations) [24-25]. However, the cohort studies that were reviewed included only a few patients (6.7%) who had received bedaquiline for

more than six months; hence, the findings provide no generalisable information about the extended use of bedaquiline [24].

Rationale

The literature review highlighted the need to evaluate the role and effect of bedaquiline use for different categories of TB patients. Exploration of factors that influence the efficacy of bedaquiline is necessary. Identifying bedaquiline gene mutations requires routine drug susceptibility testing, which may not be feasible at the programme level in settings with limited resources. In addition, there is currently no adequate protocol for testing bedaquiline susceptibility[16]. With widespread bedaquiline use, the potential for selection of DR strains indicates the need to ensure an optimal regimen and treatment length. Previously treated TB patients may harbour resistant strains because of genetic mutations, and they may have relatively high bacterial loads due to extensive parenchymal damage.

To limit bedaquiline resistance, information is needed on the profiles of patients who require extended duration of bedaquiline therapy – beyond 6 months in the absence of evidence from clinical trials, the cohort-reviewed data in this study provides pragmatic evidence for effective bedaquiline treatment duration in patients previously exposed to TB treatment. We investigated whether previous exposure to TB treatment influenced the time to culture conversion, compared with no previous TB treatment exposure, for patients who received a DR-TB regimen containing bedaquiline. We undertook the comparison by reviewing the time to culture conversion, evaluating treatment regimens, comparison of the rate of relapse, comparison of duration of bedaquiline therapy and establishing the severity of disease of previously treated and new DR-TB patients.

Materials and methods

Study Design

This retrospective cohort study examined data from confirmed DR-TB patients who were initiated on a DR-TB regimen containing bedaquiline from April 2016 to March 2019. The study setting was Sizwe Tropical Disease Hospital in South Africa. To account for changes in the DR-TB programme clinical guidelines in South Africa during the studied period, we evaluated drug regimens with different combinations of drugs (bedaquiline and ethionamide or bedaquiline and linezolid).

Culture conversion was used as a surrogate endpoint for treatment outcome. This approach accounted for patients who were still on treatment or had been discharged from the hospital to ambulatory care at local treatment facilities at the time of the analysis.

Study population

Sizwe Tropical Disease Hospital is located in Gauteng and serves as a referral centre for complicated MDR and XDR-TB cases in this province. Only patients with bacteriologically confirmed DR-TB on a regimen that contained bedaquiline, with documented culture

conversion, were included in the study. The statistics for Sizwe Hospital for the study period indicated that 1 174 DR-TB patients were initiated onto a TB regimen containing bedaquiline. Among them, 951 patients were treated for MDR-TB, 151 were treated for pre-XDR-TB and 72 were treated for XDR-TB. From incidence estimates in the literature, we anticipated that the ratio for new DR-TB patients to previously treated DR-TB patients would be 1:4 [26].

Measures

Explanatory variables:

Drug-resistant profiles of patients were defined using the DR-TB case definitions as per the South African National Department of Health [26]. An MDR-TB case was any “patient with bacteriologically proven TB with resistance to Rifampicin and Isoniazid with or without resistance to other first-line anti-TB drugs” [26]. A pre-XDR-TB case was defined as “MDR-TB with additional resistance to either a second-line injectable or a fluoroquinolone” [25]. An XDR-TB case was defined as “MDR-TB that also has resistance to at least a fluoroquinolone and one second- line injectable (Amikacin, Kanamycin and/or Capreomycin)” [25].

Patients were categorised into “new” and “previously treated” groups based on their exposure to anti-TB treatment. The new group comprised DR-TB patients who had not received any treatment or who had received treatment for TB, MDR-TB or XDR-TB for less than a month. The previously treated group comprised patients who had been treated with first-line drugs for a month or longer, or who had been treated with at least one second-line drug, either with or without first-line drugs for a month or [25].

Severity of disease was classified according to sputum smear grading, baseline chest X-ray (CXR) findings and baseline resistance patterns [25;27]. Further information on the severity of disease is provided in S1_Protocol pdf.

The categorization of patients based on their drugs exposure was described as follows: Linezolid only, Ethionamide only, no Ethionamide or Linezolid or Ethionamide and Linezolid. This categorization ensured that there was no overlap and that each category was considered independently. The combined variable ensured that the effect of both the drugs could be evaluated.

Treatment regimens assessed were reflective of current and previous South African National TB Guidelines. Patients received either a standardised long or short DR-TB bedaquiline-containing regimen [25]. Other variables that could contribute to time to culture conversion were captured from patient records. These included sociodemographic information, medical conditions, HIV status and antiretroviral therapy (ART).

Outcome variables:

We considered sputum culture conversion, time to culture conversion and duration of bedaquiline treatment as clinical endpoints for this study. Details of the definitions for

outcome variables is provided in S1_Protocol pdf.

Statistical Considerations

Sample size and power

We calculated the required sample size using WinPepi software (Version 11.65), set to detect a moderate difference between the two groups. For this purpose, we used the median survival times for 180 days in the previously treated group and 100 days in the new-patient group. These timeframes were based on the worst-case scenario described by Borisov et al. [25].

The required sample sizes were determined to be 76 for the previously treated group and 304 for the new-patient group. Hence, a total sample of 380 patients was needed to achieve power of 80% with hazard ratio of 0.556 and a significance of $\alpha = 0.05$. Details on the sample size calculation are provided in S1_Protocol pdf. Patient records were systematically and randomly sampled, with every second patient record being selected until the required sample size for each group was achieved.

Statistical analysis

We used a data extraction form (see S2_CRF 001 pdf) to collect sociodemographic, clinical and laboratory information for each patient. Patient characteristics were summarised using frequencies and percentages for categorical variables and median and interquartile ranges for continuous variables. Chi-squared and Fischer exact tests were used to evaluate associations between the category of patient and the categorical outcomes.

Time to initial conversion of sputum culture was analysed using a Kaplan-Meier survival curve, and the log rank test was used to test the difference between the groups. Time to culture conversion was truncated at 12.5 months. Only one patient was affected by this decision; this patient had received extended treatment due to treatment interruptions. Univariate logistic regression was used to assess crude associations between risk factors and the outcome of culture conversion after six months. The cut-off point of six months was derived from the methods employed in previous studies assessing bedaquiline efficacy [2, 24]. Variables associated with the outcome at the 0.2 level of significance or were known from the literature to be clinically important factors for delayed culture conversion were used in multivariable logistic regression analysis to assess independent associations with the outcome of culture conversion after six months. A p-value less than or equal to 0.05 was considered statistically significant, and the analysis was performed with STATA software (version 15.0; StataCorp, Texas 2019).

Ethical considerations

This study received ethical approval from the Human Research Ethics Committee of Stellenbosch University (S19/09/177) (S3_HREC clearance pdf). Informed consent was not

required from patients. Institutional permission was received from the chief executive officer of Sizwe Tropical Disease Hospital (S4_Institutional permission pdf) to conduct the study and access patient records.

Results

Baseline characteristics

This retrospective cohort study was undertaken for the period April 2016 to March 2019. In total, 552 files were retrieved, and 397 files were selected for auditing. Of the 155 files that were excluded, 46 lacked baseline culture information or had no documented culture conversion, 48 were cases of disseminated TB or extrapulmonary TB, two were drug-sensitive TB, one was mixed strain and 58 did not receive bedaquiline. Of the audited files, 303 were new DR-TB patients and 94 were previously treated DR-TB patients. The 94 patients in the previously treated group consisted of 68 patients with prior exposure to first-line TB drugs and 26 who had received second-line TB drugs.

The profiles of patients according to their exposure groups are shown in Table 1. Age was not stated in one file of the previously treated group. The mean age was similar for both groups, and a male predominance was noted in the previously treated group (70.2%). On comparison of the body mass index (BMI) of both groups, 47.7% of previously treated patients were found to have a BMI of less than 18.5, whereas 49.5% of the new-patient group had BMIs between 18.5 and 25. This difference in BMI was not statistically significant.

Information on comorbidities was not stated in two files of the previously treated group and one file of the new group. A total of 202 (27-previously treated and 175-new patients) had documented comorbidities Hearing loss was the predominant comorbidity for this cohort, at 41.8% (165 patients out of the 394). This point is reflective of the adverse effect of kanamycin that warranted referral to Sizwe Tropical Disease Hospital.

HIV status was known for all patients, and both groups had achieved successful ART initiation. Previous ART history was not stated in one file of the new-patient group. Modification of the ART regimen was documented for 68.3% (210/294) of those already on ART. Information on viral load was not noted in 10 files of the previously treated group and 10 files of the new-patient group. The viral load of < 400 was used as the cut-off to indicate viral suppression as per the updated South African ART guidelines, 2015 [28]. Less than a third of patients were virologically suppressed: 31.8% in the previously treated group and 29.3% in the new-patient group.

We reviewed the treatment outcomes. A substantial proportion of the new group (76.5%) had achieved successful treatment outcomes. In the previously treated group, 24.4% of patients were still being treated and there was no outcome assigned to those cases at the time of the analysis. Of the four patients with treatment failure, three from the previously treated group had received extended duration of bedaquiline.

Table 1 Demographics and clinical characteristics

Characteristics	Previously treated group	New-patient group	p-value

Age	38.27 (± 11.29) years	37.02 (± 11.78) years	0.579
Age Groups (years)			0.732
<18	4/93 (4.3%)	10/303 (3.3%)	
18-39	51 (54.8%)	171 (56.4%)	
40-59	34 (36,6%)	115 (38%)	
60-max	4 (4.3%)	7 (2.3%)	
Male	66/94 (70.2%)	160/303 (52.8%)	0.003
BMI (kg/m^2)			0.053
<18.5	42/88 (47.7%)	103/291 (35.4%)	
18.5 to <25	39/88 (44.3%)	144/291 (49.5%)	
25 to <30	2/88 (2.3%)	28/291 (9.6%)	
≥30	5/88 (5.7%)	16/291 (5.5%)	
Smoker	29/88 (33%)	67/289 (23.2%)	0.065
Comorbidities	27/92 (29.4%)	175/302 (58%)	
Diabetes Mellitus	3/27 (11.5%)	21/175 (12%)	0.894
Hypertension	5/27 (19.2%)	16/175 (9.1%)	0.137
Renal	2/27 (7.7%)	6/175 (3.4%)	0.324
Hearing Loss	20/27 (74.0%)	145/175 (82.9%)	0.358
HIV	76/94 (80.9%)	219/303 (72.3%)	0.097
On Antiretroviral Therapy (ART)	76/76 (100%)	219/219 (100%)	
Change in Regimen	52/76 (68.4%)	149/218 (68.3%)	
Viral Load <400 (copies /mL)	21/66 (31.8%)	61/208 (29.3%)	
Drug Resistance Profile			0.077
MDR	63/94 (67%)	239/303 (78.8%)	
Pre-XDR (second- line injectable drug resistance)	5/94 (5.3%)	13/303 (4.2%)	
Pre-XDR (fluoroquinolone resistance)	19/94 (20.2%)	32/303 (10.5%)	
XDR	7/94 (7.4%)	19/303 (6.2%)	
Treatment Outcomes			
Cured	27/94 (28.7%)	122/303 (40.2%)	
Treatment Complete	17/94 (18%)	110/303 (36.3%)	
Lost to Follow Up	9/94 (9.5%)	29/303 (9.5%)	
Treatment Failure	4/94 (4.2%)	0	
Died	12/94 (12.7%)	18/303 (5.9%)	
Still on Treatment	23/94 (24.4%)	10/303 (3.3%)	
Not Evaluated	0	6/303 (1.9%)	

Transferred out	2/94 (2.1%)	8/303 (2.6%)	

Time to culture conversion

The Kaplan-Meier plot (Fig 1) shows that up to two months into treatment, the two groups experienced similar rates of culture conversion. Thereafter, the new-patient group experienced faster times to culture conversion. The previously treated and new groups almost all experienced culture conversion by Month 12. The median time to culture conversion for the previously treated group was 45 days (95%CI: 34–62 days) and for the new group 53 days (95%CI: 39–62 days). The time to culture conversion for the groups was compared using the log rank test, and the difference was not statistically significant (p= 0.278). However, it was noted that the proportions culture converting by group were not proportional over time.

Since all patients eventually achieved culture conversion, the time to culture conversion was split into two categories: i) culture converted in six months or less or ii) longer than six months. There was a statistically significant (p=0.004) higher proportion of culture conversion by six months of treatment for the new group (296/303; 97.7%) compared with the previously treated group (85/94; 90.4%).

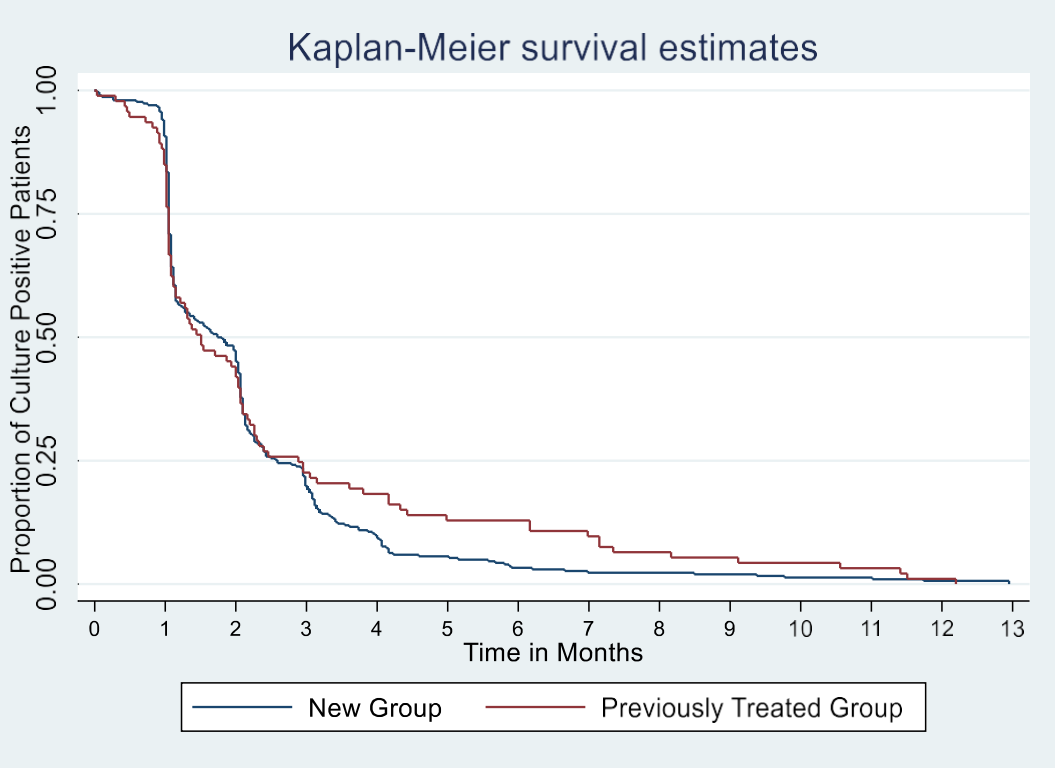

Figure 1: Kaplan-Meier plot for time to culture conversion for new and previously treated groups (N=397)

Due to the small numbers, smear grade scanty and Grade 1+ were combined for analysis (Table 2). The univariate analysis showed that only previously treated category of patient attained statistical significance but other variables of clinical significance such as age group <18 years, linezolid-containing regimen and smear grade 3+ showed an increased odds of prolonged time to culture conversion when compared to the reference category. On review of outcomes from the multivariable analysis (Table 2), the previously treated group displayed 4.12 times the adjusted odds of delayed culture conversion compared with the new-patient group. In addition, although the difference was not statistically significant, patients younger than 18 years had 4.89 times the adjusted odds of longer time to culture conversion than patients aged 18 years or older (p=0.074). Furthermore, a smear grade of +3 was shown to have 2.02 times the adjusted odds of delayed time to culture conversion than a negative smear grade, although the difference was not statistically significant (p=0.263). The use of a linezolid containing regimen was independently affected by various characteristics, with a change in the odds ratio on addition of other variables into the model (Table 2).

Table 2: Odds ratios: time to culture conversion after 6 months and risk factors (univariate and multivariable models)

Characteristic	**Odds Ratio (95% CI); p-value**	
Category of patient	Univariate models	Multivariable model
New	1	1
Previously Treated	4.48 (1.62–12.38); 0.004	4.12 (1.45–11.72); 0.008
Resistance Profile		
MDR	1	
Pre-XDR (second line injectable drug resistance)	1.21 (0.15–9.75); 0.858	
Pre-XDR (fluroquinolone resistance)	0.41 (0.05–3.20); 0.396	
BMI (kg/m^2)		
< 18.5	1	
18.5 to <25	1.82 (0.55–6.04); 0.326	
25 to <30	2.52 (0.44–14.42); 0.300	
>30	1.76 (0.19–16.57); 0.620	
Gender		
Female	1	
Male	1.03 (0.38–2.82); 0.955	
Age Group (years)		
18-39	1	1
<18	3.2 (0.64-16.07); 0.636	3.66 (0.63-21.28); 0.631
40-59	0.26 (0.06-1.20); 0.084	0.27 (0.06-1.24); 0.093
60-max	1.92 (0.23-16.36); 0.551	1.68 (0.18-16.01); 0.650
HIV Status		
HIV negative	1	
HIV positive	0.75 (0.25–2.22); 0.605	
Regimen		
Non linezolid-containing regimen	1	1
linezolid-containing regimen	2.13 (0.67–6.71); 0.199	1.53 (0.46–5.10); 0.489
Non ethionamide-containing regimen	1	
ethionamide-containing regimen	1.29 (0.44–3.80); 0.641	
Smear Grade		
Negative	1	1
Scanty /Grade 1+	0.99 (0.23–4.25); 0.708	1.23 (0.28–5.48); 0.785
Grade 2+	1.22 (0.23–6.46); 0.819	0.94 (0.16–5.54); 0.947
Grade 3+	2.10 (0.62–7.07); 0.233	2.05 (0.58–7.19); 0.263
CXR		
No cavity	1	
Single cavity	1.60 (0.52–4.91); 0.412	

Multi-cavitatory	0.47 (0.06–3.75); 0.473	

Background regimen

For this cohort, the previously treated group mainly received a linezolid-containing regimen (41.4%), whereas the new group received an ethionamide containing regimen (34.9%)(p=0.001) (Table 3). Further analysis showed that 33/48 (66.6%) of patients on linezolid (300 mg), 123/159 (77%) of patients on linezolid (600 mg) and 16/19 (84%) of patients on linezolid (1200 mg) achieved culture conversion within three months of starting treatment. Highly similar rates of relapse were found between the previously treated (3/93; 3.2%) and new-patient groups (9/302; 3%) (p=0.904). Twelves cases of relapse during treatment were noted, of whom five patients were receiving bedaquiline at the time of relapse.

Table 3: Clinical evaluation by group and drug-containing regimen (N=397)

Category	**no linezolid or ethionamide**	**linezolid only**	**ethionamide only**	**linezolid and ethionamide**	**p-value**
Previously Treated	6/94 (6.4%)	39/94 (41.5%)	18/94 (19.2%)	31/94 (33%)	0.001
New	32/303 (10.6%)	69/303 (22.8%)	106/303 (35%)	96/303 (31.7%)	

Duration of bedaquiline treatment

Only 344 patients with a documented duration of bedaquiline therapy were included in the analysis. A higher proportion of the previously treated group received a longer duration of bedaquiline than the new group (27/75 vs 42/269 or 36% vs 15% (≥eight months of treatment); p<0.001). The results are summarised in Table 4. Additionally the median duration of bedaquiline for the new group was 183 days and for the previously treated group 188 days (p=0.035).

Table 4: Duration of bedaquiline treatment by category of patient (N=344)

Category	**6 months**	**7 months**	**8 months**	**9 months**	**p-value**
New	108/269 (40.2%)	119/269 (44.2%)	24/269 (8.9%)	18/269 (6.7%)	<0.001
Previously treated	23/75 (30.7%)	25/75 (33.3%)	10/75 (13.3%)	17/75 (22.7%)	

Evaluation of the 52 patients (28 new; 24 previously treated) for whom bedaquiline treatment was interrupted indicated that bedaquiline treatment was permanently stopped for only six patients (two new and four previously treated). Reasons for treatment interruption were primarily transient QTc interval prolongation (25/52 or 48%) and drug-induced liver injury or transaminitis (24/52 or 46.1%).

Severity of disease

Previously treated patients were shown to have more severe disease than new patients, based on CXR findings. Cavitatory disease was significantly associated with previous TB disease (p=0.006) (Table 5). A subgroup analysis of inhA resistance pattern in absence of KatG mutation was performed to evaluate for any between- group differences. No significant difference in proportion of inhA mutation was found on comparison of the two groups (p=0.377). The previously treated group had higher proportion of the gyra A /gyra B gene mutation, indicating resistance to fluoroquinolones, as compared to the new group (p=0.036). (Table 5).

Table 5: Severity of disease according to category of patient

Severity of Disease	**Previously Treated**	**New**	**p-value**
Baseline Smear Grade			0.683
Negative	40/94 (42.6%)	117/303 (38.6%)	
Scanty	3/94 (3.2%)	20/303 (6.6%)	
Grade 1+	15/94 (16%)	57/303 (18.8%)	
Grade 2+	12/94 (12.8%)	40/303 (13.2%)	
Grade 3+	24/94 (25.5%)	69/303 (22.8%)	
CXR			0.006
No Cavitation	45/92 (48.9%)	203/302 (67.3%)	
Single Cavitation	28/92 (30.4%)	60/302 (19.9%)	
Multiple Cavitations	19/92 (20.7%)	39/302 (12.9%)	
Resistance Patterns			
inhA alone with no KatG mutation (n=188)	9/44 (20.5%)	39/144 (27.1%)	0.377
KatG with inhA mutation	50/94 (53.1%)	158/303 (52.1%)	0.859
RpoB	89/94 (94.6%)	301/303 (99.3%)	0.003
SLID	11/94 (11.7%)	32/303 (10.5%)	0.731
gyra A/gyra B	25/94 (26.5%)	51/303 (16.8%)	0.036

Discussion

This study was undertaken mainly to determine whether previous TB treatment as compared to no previous TB treatment, influenced the time to culture conversion for DR-TB patients on a bedaquiline-containing regimen. Our findings indicate that while there was no statistically significant difference between the two groups in terms of time to event analysis, when the time to culture conversion was dichotomised at six months, the previously treated group had a significantly higher probability of culture converting after six months than the new group. Further analysis of the factors that influenced the time to culture conversion revealed that the odds of culture conversion failure at six months was four times higher among previously treated patients than new patients.

We also found that the resistance profiles, HIV status, gender and the severity of disease did not influence the time to culture conversion. We did not anticipate that HIV status would be a risk factor, as all HIV positive patients in this cohort received a modified ART regimen once initiated on bedaquiline. The adverse effects of efavirenz in reducing steady state concentrations of bedaquiline was mitigated. Thus optimal drug concentrations of bedaquiline were ensured [29]. Although a large proportion of the previously treated group were male, in keeping with the literature findings [24], gender did not influence time to culture conversion. A similar finding was shown in a previous study, which found no differences in culture conversion times among male and female DR-TB patients [30]. We found that patients younger than 18 years had higher odds of prolonged time to culture conversion although this result did not reach statistical significant. Harausz et al, in a systematic review of treatment outcomes of MDR-TB children found that they were more inclined to be older, HIV-infected and with cavitatory TB [31]. Thus, our finding does require further exploration.

The median time to achieve culture conversion was similar for both groups up to the two-month mark, with the majority achieving culture conversion. This outcome is explained by the theory on the exposure–response relationship of bedaquiline in DR-TB. High-level bedaquiline exposure was found to have a beneficial effect within the first 20 weeks of treatment [32]. Bedaquiline has been shown to eliminate persistent TB bacilli as early as four weeks into treatment [33].

For this study , most of the patients who received extended duration of bedaquiline treatment were in the previously treated group. A greater severity of disease was also noted in the previously treated group, which may have prompted clinicians to extend the duration of bedaquiline treatment until culture conversion. Most new patients in this study cohort received bedaquiline for the recommended duration of six months [4]. An earlier study [32] also showed that previously treated patients experienced a longer median time (up to four weeks longer) to culture conversion and required a longer duration of bedaquiline treatment to achieve conversion, compared with new patients. Guglielmetti et al. [34] similarly found that extended use of bedaquiline (beyond six months) was noted for previously treated XDR-TB, but the duration was not significantly associated with time to culture conversion. However, we established a significant association for the previously treated group, irrespective of drug resistant profile, regarding extended bedaquiline use and prolonged time to culture conversion. This was because our study was designed to detect a moderate difference between the two patient categories with sufficient statistical power.

Despite the efficacy of bedaquiline treatment, we found 12 cases of treatment relapse and three treatment failures among previously treated patients who received extended duration bedaquiline. This outcome suggests that bedaquiline resistance may have been acquired by these patients, but further exploration is required. A retrospective study that examined MIC and culture conversion found that 75% of patients with prior bedaquiline exposure who failed to achieve culture conversion within six months displayed an Rv0678 mutation [35]. Thus, for patients who receive extended bedaquiline treatment, especially previously treated TB patients, MIC may have to be monitored to ensure susceptible bedaquiline MIC. As described in a recent study amongst HIV infected DR-TB patients, an association between emergent Rv0678 variants, failure to culture convert at 6 months and raised bedaquiline MICs was a notable finding [36].

Our analysis of the category of smear grade, the CXR findings and the gene mutations confirmed greater severity of disease among previously treated patients than new patients. The high percentage of fluoroquinolone resistance, in comparison to second line injectables, found in this study has also been documented previously [34]. Despite our finding of greater severity of disease in the previously treated group, this feature had no statistical significant influence on culture conversion. The association between severe disease and longer time to culture conversion was documented in earlier studies [30,37]. The failure to demonstrate a difference in this study may be explained by the study setting ., only patients with severe TB disease are referred to Sizwe Tropical Disease Hospital for further management.

In both groups, treatment was interrupted because of adverse effects of bedaquiline therapy; however, the interruptions did not adversely influence the median duration of bedaquiline treatment. This finding is congruent with previous evidence that bedaquiline toxicity was infrequent and was reversible [2,24,38]. Pontali et al. found that QTc interval prolongation was common but reverted to baseline after a week [2].

The WHO guidelines advise using an optimal background regimen with bedaquiline, which includes linezolid and a fluoroquinolone [39]. In our study, most patients in the previously treated group received a linezolid-containing regimen. This finding may be partially explained by the change in the TB programme guidelines. In both groups, a high dosage of linezolid was associated with a high proportion of patients' culture-converting within three months. The use of high dose (1200 mg) linezolid was examined in the Nix-TB trial, which showed that 90% of patients with highly drug-resistant TB achieved successful treatment outcomes [40]. We found no significant association between linezolid inclusion in a regimen and the time to culture conversion. The benefit of including linezolid in the background regimen was confirmed by the number of previously treated patients who achieved early culture conversion – at rates comparable to those of new patients. This postulation is supported by the findings of a previous study, in which the rate of culture conversion was twice as high in patients receiving a bedaquiline and linezolid regimen as compared to the standard regimen [41].

The retrospective design of our study implies some limitations. First, missing information may have affected the quality of our data, but efforts were made to review source documents for completeness. Second, Sizwe Tropical Disease Hospital serves as a referral hospital for severe forms of TB disease, and referral for initiation of bedaquiline treatment due to hearing loss was a criterion for our sample. Thus our findings cannot be extrapolated to primary health facilities that mainly treat ambulatory MDR-TB patients.

Third, correlational analysis of the duration of bedaquiline treatment and clinical outcomes was not performed due to 24.4% of patients in the previously treated group still being on treatment. This may be reflective of the time frame for treatment initiation, which may have occurred later than patients in the new group. Because not all patients in the study had an outcome assigned, we relied on the surrogate endpoint of culture conversion to establish the efficacy of bedaquiline.

Fourth, the South African National TB Guidelines underwent several changes over the study period. Our study reflects both the short and long course regimens but accounts only for the use of linezolid and ethionamide in these regimens. Hence, the impact of other newer agents was not examined. Furthermore, documentation of MICs for bedaquiline was not a feature of the TB programme, which meant we could not analyse the influence of bedaquiline's MIC regarding culture conversion. Such an analysis may have been useful for

reviewing the extended duration of bedaquiline treatment. However, despite the above limitations we were able to provide pragmatic evidence on the programmatic implementation of bedaquiline and the influence of previous TB treatment.

Conclusion

To our knowledge, this is the first study to compare time to culture conversion across categories of DR-TB patients. We established that among patients receiving a bedaquiline-containing regimen, previously treated patients had four times the odds of delayed culture conversion than new patients. Most previously treated DR-TB patients who failed to culture convert early (by two months) were exposed to a longer duration of bedaquiline and experienced longer times to culture conversion than new DR-TB patients. For those in the previously treated group who achieved early culture conversion, inclusion of linezolid at higher doses in the regimen might have contributed to the outcome.

The findings of this study contribute to the literature by exploring the potential impact of previous exposure to TB treatment and establishing the influence on the efficacy of current bedaquiline therapy. Given the extended use of bedaquiline, monitoring of susceptible bedaquiline MIC in previously treated patients as early as two months in the event of failure to culture convert may be warranted. In addition further evaluation of other prescribed concurrent medication which may affect bedaquiline MIC would also need to be undertaken. Such monitoring can help prevent emergent bedaquiline drug resistance.

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Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., “We used the techniques developed by our colleagues [19] to analyze the data”). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order before ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the “Vancouver” style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source Format

Published articles

Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588.

Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005.

Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.

Accepted, unpublished articles Same as published articles, but substitute "Forthcoming" for page numbers or DOI.

Online articles

Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14

Books

Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.

Book chapters Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.

Deposited articles (preprints, e-prints, or arXiv)

Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1

Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278

Published media (print or online newspapers and magazine articles) Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html

New media (blogs, web sites, or other written works) Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2 screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.

Masters' theses or doctoral dissertations Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09

Databases and repositories (Figshare, arXiv) Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214

Multimedia (videos, movies, or TV shows) Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an “S” and number. For example, “S1 Appendix” and “S2 Appendix,” “S1 Table” and “S2 Table,” and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

Figures and tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

Read the guidelines for figures and requirements for reporting blot and gel results.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

A figure label with Arabic numerals, and “Figure” abbreviated to “Fig” (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of “Fig 1” must refer to a figure file named “Fig1.tif”).

A concise, descriptive title

The caption may also include a legend as needed.

Read more about figure captions.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., “Table 1”) and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Read the guidelines for tables.

Statistical reporting

Manuscripts submitted to PLOS ONE are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously reported in accordance with community standards and that the statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

SAMPL guidelines, for general guidance on statistical reporting

PLOS ONE guidelines, for clinical trials requirements

PLOS ONE guidelines, for systematic review and meta-analysis requirements

EQUATOR, for specific reporting guidelines for a range of other study types

Reporting of statistical methods

In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

List the name and version of any software package used, alongside any relevant references

Describe the technical details or procedures required to reproduce the analysis

Provide the repository identifier for any code used in the analysis (See our code-sharing policy.)

Statistical reporting guidelines:

Identify research design and independent variables as being between- or within-subjects

For pre-processed data:

Describe any analysis carried out to confirm the data meets the assumptions of the analysis performed (e.g. linearity, co-linearity, normality of the distribution).

If data were transformed include this information, with a reason for doing so and a description of the transformation performed

Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed

If relevant, describe how missing/excluded data were handled

Define the threshold for significance (alpha)

If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.

For analyses of variance (ANOVAs), detail any post hoc tests that were performed

Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so

Describe all options for statistical procedures. For example, if t-tests were performed, state whether these were one- or two-tailed. Include details of the type of t-test conducted (e.g. one sample, within-/between-subjects).

For step-wise multiple regression analyses:

Report the alpha level used

Discuss whether the variables were assessed for collinearity and interaction

Describe the variable selection process by which the final model was developed (e.g., forward-stepwise; best subset). See SAMPL guidelines.

For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

Reporting of statistical results

Results must be rigorously and appropriately reported, in keeping with community standards.

Units of measurement. Clearly define measurement units in all tables and figures.

Properties of distribution. It should be clear from the text which measures of variance (standard deviation, standard error of the mean, confidence intervals) and central tendency (mean, median) are being presented.

Regression analyses. Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.

Reporting parameters. Test statistics (F/t/r) and associated degrees of freedom should be provided. Effect sizes and confidence intervals should be reported where appropriate. If percentages are provided, the numerator and denominator should also be given.

P-values. Report exact p-values for all values greater than or equal to 0.001. P-values less than 0.001 may be expressed as $p < 0.001$, or as exponentials in studies of genetic associations.

Displaying data in plots. Format plots so that they accurately depict the sample distribution. 3D effects in plots can bias and hinder interpretation of values, so avoid them in cases where regular plots are sufficient to display the data.

Open data. As explained in PLOS's Data Policy, be sure to make individual data points, underlying graphs and summary statistics available at the time of publication. Data can be deposited in a repository or included within the Supporting Information files.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

See instructions on providing underlying data to support blot and gel results

Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

Deposit data in the integrated repository of choice.

Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.

Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

Ensembl

Entrez Gene

FlyBase

InterPro

Mouse Genome Database (MGD)

Online Mendelian Inheritance in Man (OMIM)

PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a “Striking Image” that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

Additional Information Requested at Submission

Financial Disclosure Statement

This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

Specific grant numbers

Initials of authors who received each award

Full names of commercial companies that funded the study or authors

Initials of authors who received salary or other funding from commercial companies

URLs to sponsors' websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

Study design

Data collection and analysis

Decision to publish

Preparation of the manuscript

If they had no role in the research, include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."

If the study was unfunded, include this sentence as the Financial Disclosure statement: "The author(s) received no specific funding for this work."

Read our policy on disclosure of funding sources.

Competing interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

Read our policy on competing interests.

Manuscripts disputing published work

For manuscripts disputing previously published work, it is PLOS ONE policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to PLOS ONE or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into “parts.” Each submission to PLOS ONE must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to PLOS ONE, the authors may be advised to combine them into a single manuscript at the editor's discretion.

Read our policies on related manuscripts.

Preprints

PLOS encourages authors to post preprints as a way to accelerate the dissemination of research and supports authors who wish to share their work early and receive feedback before formal peer review. Deposition of manuscripts with preprint servers does not impact consideration of the manuscript at any PLOS journal.

Authors posting on bioRxiv or medRxiv may submit directly to relevant PLOS journals through the direct transfer to journal service.

Authors submitting manuscripts in the life sciences to PLOS ONE may opt-in to post their work on bioRxiv during the PLOS ONE initial submission process.

Read more about preprints.

Learn how to post a preprint to bioRxiv during PLOS ONE initial submission.

Guidelines for Specific Study Types

Registered Reports

Submission and format requirements for Registered Report Protocols and Registered Reports are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

Provide enough methodological detail to make the study reproducible and replicable

Confirm that data will be made available upon study completion in keeping with the PLOS Data policy

Include ethical approval or waivers, if applicable

Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle

For meta-analyses or Clinical Trials, use the protocol-specific reporting guidelines PRISMA-P or SPIRIT respectively

For more guidance on format and presentation of a protocol, consult the sample template hosted by the Open Science Framework. Discipline-specific and study-specific templates are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.

The manuscript may also contain exploratory, unplanned analyses.

Read more about Registered Report framework.

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of

written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form from the Downloads and Translations page. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

Studies of diagnostic accuracy: STARD

Observational studies: STROBE

Microarray experiments: MIAME

Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

The name of the approving institutional review board or equivalent committee(s). If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed

Whether informed consent was written or oral. If informed consent was oral, it must be stated in the manuscript:

Why written consent could not be obtained

That the Institutional Review Board (IRB) approved use of oral consent

How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

Explicitly describe their methods of categorizing human populations

Define categories in as much detail as the study protocol allows

Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency

Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about PLOS ONE policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. PLOS ONE follows the World Health Organization's (WHO) definition of a clinical trial:

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. Where trials were not publicly registered before participant recruitment began, authors must:

Register all related clinical trials and confirm they have done so in the Methods section

Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

The name of the registry and the registration number must be included in the Abstract.

Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.

Include the CONSORT flow diagram as the manuscript's “Fig 1”

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The name of the registry and the registry number must be provided in the Abstract. If the trial is registered in more than one location, please provide all relevant registry names and numbers.

Lab Protocols

Lab Protocols consist of two interlinked components: a protocol hosted on the protocols.io platform and a peer-reviewed article on PLOS ONE that contextualises the protocol.

protocols.io is a secure open access platform that specializes in laboratory protocols. It allows scientists to share, discover and reuse up-to-date protocol knowledge. The platform provides specialist tools and guidance on how to add each element of the protocol, including the title, abstract, steps, files, links, reagents, measurements, formulae, videos, charts and more.

The PLOS ONE article component must comply with the general submission guidelines (detailed above in this article).

The PLOS ONE article component must also comply with the general PLOS ONE criteria for publication and in addition it should:

Present a step-by-step protocol that adds value to the published literature.

Link, in the Introduction section, to at least one supporting peer-reviewed publication in which the protocol was applied to generate data.

Link, in the Materials and Methods section, to the protocol.io component, using the digital object identifier (DOI) and format provided by protocols.io, for example https://dx.doi.org/10.17504/protocols.io[...].

Describe the appropriate controls, sample sizes and replication needed to ensure that the data are robust and reproducible.

Provide the protocol as a supporting information (S1) file for printing purposes. You can download a PDF from protocols.io for this purpose.

Optionally, provide minimal new data relevant to the development of the protocol e.g., for additional benchmarking, validation or troubleshooting purposes.

Download a sample Lab Protocol template

Lab Protocols describing routine methods, or extensions or modifications of routine methods, add little or no value to the published literature and will not be considered for publication.

Manuscripts that report new methods should be submitted as research articles, not as Lab Protocols

Lab Protocols are subject to the same editorial and peer review process as all other articles, except that the peer review process may be expedited and carried out by one internal Academic Editor and one external reviewer.

Lab Protocols are eligible for both signed and published peer review.

We encourage you to post your protocol to the protocols.io platform before submitting your manuscript to PLOS ONE, or at the latest, before the editorial and peer review process. This approach is optional, but beneficial, because:

Your DOI is assigned on the protocols.io platform. You need this identifier to link out from the Material and Methods section of your manuscript.

You can keep your protocol private on the protocols.io platform (until you are satisfied that it is ready for publication), but still assign a DOI.

The protocol will be accessible to editors and reviewers during the editorial and peer review process.

If you prefer to submit your manuscript to PLOS ONE before uploading your protocol to protocols.io, please provide your protocol as a supporting information (S1) file. You can use protocols.io's editorial service at no cost: they will check and publish your protocol for you t. As part of PLOS ONE's partnership with protocols.io, your waiver code for this purpose will be provided in the first decision letter.

Preprint posting is not available for Lab Protocols and bioRxiv does not accept them.

Study Protocols

Study Protocols describe plans for conducting research projects and consist of a single article on PLOS ONE.

Study Protocols must comply with the PLOS ONE general submission guidelines (detailed above in this article) and any guidelines specific to the related research study type. In addition, the protocol must:

Relate to a research study that has not yet generated results.

Be submitted before recruitment of participants or collection of data for the study is complete.

Meet the same standards for ethics of experimentation and research integrity as the research study. If it involves human or animal subjects, cell lines or field sampling, or has potential biosafety implications, prior approval from the relevant ethics body must be obtained prior to submission. Please contact us if you have a valid reason for not obtaining approval.

Additional prerequisites apply for these study types:

Clinical trials:

The trial must be registered prior to submission of your protocol in one of the publicly accessible registries approved by the WHO or ICMJE (Internation Committee of Medical Journal Editors).

The name of the registry and the trial or study registration number must be included in the Abstract.

A copy of the protocol that was approved by the ethics committee must be submitted as a supplementary information file. Please provide an addtional English translation if the original document is not in English.

A SPIRIT schedule of enrollment, interventions, and assessments must be included as the manuscript's Figure 1, and a completed SPIRIT checklist must be uploaded as Supporting Information file S1.

Systematic reviews and meta-analyses:

A completed PRISMA-P checklist must be provided as a supporting information (SI) file. See PRISMA-P Explanation and Elaboration for more information on completing your checklist.

Study Protocols must also comply with general PLOS ONE criteria for publication and in addition you should:

include the word "Protocol" in your Title.

include a detailed description of the planned study in the Materials and Methods section.This should provide sufficient methodological detail for the protocol to be reproducible and replicable. Your description should cover all relevant and applicable facts and hypothesis, including:

the aim, design, and settling

the sample size calculation

how data saturation will be determined (for qualitative studies)

the characteristics of participants e.g., inclusion and exclusion criteria, sample selection criteria, variables to be measured, randomization and blinding criteria (where applicable), and how informed consent will be obtained

how materials will be selected and used e.g., where and how they will be sourced, the processes, interventions, or comparisons to be used, the outcomes to be measured, and when and how they will be measured

the data management plan

safety considerations

the type of data and statistical analyses to be used

the status and timeline of the study, including whether participant recruitment or data collection has begun

where and when the data will be made available. See our Data Availability policy for more.

include an analysis of preliminary or pilot data, only if it is necessary to support the feasibility of the study or as a proof of principle. This is optional.

we encourage authors you to register with OSF and provide the your registration number in the Materials and Methods section. This is optional.

optionally add any other SI files, figures or tables that elaborate or authenticate the protocol: e.g., any reporting checklists applicable to your study type.

Read the supporting information guidelines for more details about adding SI files.

Download our sample Study Protocol template or an OSF discipline or study-specific template.

Study Protocols are subject to the same editorial and peer review process as all other articles, and are eligible for both signed and published peer review.

You can expedite the review process by providing:

proof of external funding. This is typically your funding approval letter and a list of the names and credentials of the funders who conducted the external peer review of the protocol. Include an English translation if needed.

proof of ethics approval (if required). This is typically the approval or waiver letter from the relevant ethics body and a copy of the protocol approved by this body.

These documents are used for internal purposes and do not form part of the published Study Protocol.

Expedited review is conducted by an internal Staff Editor only and bypasses the external review process.

If the Study Protocol describes a replication study or involves re-analysis of published work, we will invite the author of the initial or replicated study to provide a signed review.

We encourage you to share your Study Protocol with other researchers, either before or after submission. You can publish it on your website or protocols.io, or submit it for posting on medRxiv or another preprint server.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

The full name of the relevant ethics committee that approved the work, and the associated permit number(s).

Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.

Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

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Definition of a humane endpoint

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Hymenogaster huthii. Stielow et al. 2010, sp. nov.
[urn:lsid:indexfungorum.org:names:518624]

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Title:

Influence of previous tuberculosis treatment history on time to culture conversion for patients receiving a Bedaquiline containing regimen at Sizwe Tropical Disease Hospital, South Africa

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Contents

1. Abstract

Tuberculosis remains one of the leading causes of death worldwide. There is a growing crisis concerning the number of drug resistant TB cases. New drug regimens were urgently needed to improve mortality and morbidity among drug resistant TB patients. Bedaqualine is a newly developed diarylquinoline with a unique mechanism of action. . Studies have reported varying time to culture conversion regarding RR/MDR-TB patients with history of previous TB treatment. The Rv0678 mutation found in patients with prior Rifampicin exposure has demonstrated a more than four-fold increase in the minimum inhibitory concentrations of Bedaquiline. Previously treated TB patients may be more likely to have higher bacterial load due to extensive parenchymal damage. This study will provide information on effective Bedaquiline treatment duration for those previously exposed to TB treatment.

Aim: To determine whether previous exposure to TB treatment influences the time to culture conversion as compared to no previous TB treatment exposure in patients receiving a DR-TB regimen containing Bedaquiline.

Primary Objective: To compare the time to culture conversion for previously treated and new DR-TB patients receiving the Bedaquiline containing regimen.

Secondary Objectives:

1. To evaluate treatment regimens at time of culture conversion for previously treated and new DR-TB patients

2. To compare the rate of relapse in previously treated and new DR-TB patients receiving the Bedaquiline containing regimen.

3. To compare the duration of Bedaquiline therapy in previously treated and new DR-TB patients

4. To establish the severity of disease of previously treated and new DR-TB patients.

Setting and Study Population: The study will be conducted at Sizwe Tropical Disease Hospital in Gauteng Province. Sizwe Tropical Disease Hospital serves as a referral centre for complicated MDR/XDR-TB cases in Gauteng.

Study Design: A retrospective cohort study will be undertaken for confirmed DR-TB patients who were initiated on DR-TB regimen containing Bedaquiline from April 2016 to March 2019.

Inclusion Criteria

Bacteriologically confirmed DR-TB

All patients receiving DR-TB Regimens containing Bedaquiline including new DR-TB with no previous history of TB treatment

Documented culture conversion

Variables: Culture Conversion; Time to culture conversion; Duration of Bedaquiline treatment

Sampling Technique:

The group sample sizes were determined to be 76 (Previous treatment group) and 304 (New treatment group), power of 80% with a level of significance of α 0.05.

Time Frame: The study will be conducted from June 2020 to November2020

2. Background and Rationale

Tuberculosis remains one of the leading causes of death worldwide. There is a growing crisis concerning the number of drug resistant TB cases, 558 000-incident cases in 2017 of which Rifampicin Resistant (RR) /Multi Drug Resistant (MDR)-TB accounted for 82%.[1] It was shown that globally 3.5% of new TB cases and 18% of previously treated cases had RR/MDR-TB disease.[1] On comparison to drug-susceptible TB, MDR-TB has higher mortality and morbidity rates, with poor treatment success rate for both MDR/RR-TB and Extensively Drug Resistant (XDR)-TB globally.[1]

The low success rate in drug resistant TB was as a result of high mortality, loss to follow up and adverse drug reactions.[1] The treatment of MDR-TB and XDR-TB is long, expensive and with frequent side effects.[1] These are some of the reasons for poor treatment outcomes. In addition, there is difficulty in designing a suitable drug regimen with four effective drugs.[2] Thus, new drug regimens were urgently needed to improve mortality and morbidity among drug resistant TB patients.

Effectiveness of Bedaquiline

Bedaqualine is a newly developed diarylquinoline with a unique mechanism of action, specifically inhibiting mycobacterial adenosine triphosphate synthase.[3] Clinical trials have shown the benefit of reduced time to sputum conversion when Bedaquiline was included in a treatment regimen. Bedaquiline has been included as one of the drugs in drug resistant TB regimens. With the use of Bedaquiline in the current RR/MDR-TB regimen high treatment success rates (87-90%) have been achieved.[4]

The effectiveness of therapy is based on sputum culture conversion during the initial 6-month treatment period. From the results of a systematic review of the therapeutic efficacy of Bedaquiline, sputum conversion was demonstrated during the first 2 months of treatment (achieved in 48% to 84% of patients).[5] The use of Bedaquiline in treatment regimens for culture positive pulmonary MDR-TB and XDR-TB achieved culture conversion in 79% of patients after 3 months and in 97 % of patients after 6 months of treatment respectively.[6] Reduced time to sputum culture conversion has been a useful early predictor of successful final treatment outcome.

The WHO has recommended that Bedaquiline be added to an optimised background therapy of four effective second-line drugs..[7] . Much of the evidence on use of Bedaquiline for extended periods have been from studies evaluating XDR- TB treatment regimens. Duration of Bedaquiline use was shown to be successfully extended in XDR-TB with no significant adverse effects.[8] A case series report of patients with individualised XDR- TB regimens showed a benefit with the use of Bedaquiline with no adverse events of QT prolongation being recorded despite co-administration with Clofazimine and Moxfloxicin (both drugs having side effects profile of QT prolongation).[9] The major concerns regarding extended use of Bedaquiline is accumulative toxicity and risk of QT prolongation.[10]

In a systematic review conducted by Li et al, Bedaquiline was shown to have favourable TB outcomes with advanced early bactericidal activity only when combined with other bactericidal drugs.[11] Bedaquiline, thus should be accompanied by an effective background regimen in order to ensure improved treatment outcomes. Including Bedaquiline in drug regimens that are suboptimal could result in selection pressure and drug resistant mutations.[12] Several genetic targets that are associated with Bedaquiline resistance have been identified. Several studies have described the presence of the Rv0678 gene mutation on evaluating Bedaquiline therapy. [13,14,15]

The Rv0678 mutation has demonstrated a more than four-fold increase in the minimum inhibitory concentrations of Bedaquiline.[16] The description of Rv0678 mutations in isolates from MDR-TB patients without prior Bedaquiline exposure were more pronounced in MDR-TB than DS-TB patients. Villelas et al found the presence of Rv0678 mutations in patients with prior Rifampicin exposure during their evaluation of mutations and the impact on treatment outcomes.[15] Although the significance of mutations still requires further exploration, the association between prior exposure to drugs used in first line TB regimens and the presence of mutations warrants attention.

Previous treatment history influencing efficacy of Bedaquiline

It has been shown that the drug resistance profiles of Mtb isolates influences the rate of conversion, an inverse relation between conversion rate and degree of drug resistance exists.[17] Thus, previous TB treatment is regarded as an important risk factor for RR/MDR-TB. Rigorous management is required as these patients may have been infectious for a longer period.[18] When compared to new TB patients, previously treated TB patients showed a higher prevalence of drug resistance against first line anti TB drugs .[19] Studies have also shown that previous treatment history of TB is a risk factor for poor TB outcomes. [20,21] Varying time to culture conversion regarding RR/MDR-TB patients with history of previous TB treatment despite rapid bactericidal effect of Bedaquiline has also been demonstrated. [22,23]

A study by Guglielmetti et al evaluating treatment effects of Bedaquiline in MDR-TB and XDR-TB patients,showed that the time to culture conversion was adversely affected by the presence of lung cavities.[6] Relapse in patients with previous TB history may be due to fibrotic lesions or persistent cavities. A systematic review of cohort studies on the use of Bedaquiline showed that there was risk of poor outcomes in patients with lung cavitations and more severe drug resistance. [24] The cohort studies reviewed however consisted of only a few numbers of patients (6.7%) who received Bedaquiline for more than 6 months and therefore did not provide information on the role of extended use of Bedaquiline.[26]

Rationale

The literature reviewed highlights the need for evaluation of the role and the effect of Bedaquiline use with different categories of TB patients. Exploration of factors influencing efficacy of Bedaquiline is important. Identifying Bedaquiline gene mutations requires routine drug susceptibility testing which may not be feasible at a programmatic level in resource-limited settings. In addition, there is currently no adequate protocol testing Bedaquiline susceptibility in low to middle income countries.[16] With widespread Bedaquiline use, the potential for selection of drug resistant strains signifies the importance of ensuring optimal regimen selection and length of treatment. Previously treated TB patients may harbour resistant strains as a result of genetic mutations and may be more likely to have higher bacterial load due to extensive parenchymal damage. In order to limit Bedaquiline resistance, information is needed on the profile of patients requiring extended duration of

Bedaquiline therapy beyond 24 weeks. In the absence of evidence from clinical trials, the cohort-reviewed data from this study will provide pragmatic evidence on effective Bedaquiline treatment duration for those previously exposed to TB treatment. The findings of this study will contribute to the literature by substantiating the potential impact of previous exposure to TB treatment on the efficacy of Bedaquiline therapy. This study will also draw focus on the importance of the choice of background regimens when extended duration of Bedaquiline is required and the possible need for monitoring of minimum inhibitory concentrations for this subgroup.

3. Objectives and Outcomes

3.1 Research Question

In patients receiving the DR-TB regimen containing Bedaquiline, does previous exposure to TB treatment as compared to no previous TB treatment exposure, increase the time to culture conversion at Sizwe Tropical Disease Hospital from April 2016 to March 2019.

3.2 Overall Aim

To determine whether previous exposure to TB treatment influences the time to culture conversion as compared to no previous TB treatment exposure in patients receiving a DR-TB regimen containing Bedaquiline.

3.3 Primary Objectives

1. To compare the time to culture conversion for previously treated and new DR-TB patients receiving the Bedaquiline containing regimen.

3.4 Secondary Objectives

1. To evaluate treatment regimens (linezolid or ethionamide containing regimens) at time of culture conversion for previously treated and new DR-TB patients
2. To compare the rate of relapse in previously treated and new DR-TB patients receiving the Bedaquiline containing regimen.
3. To compare the duration of Bedaquiline therapy in previously treated and new DR-TB patients
4. To establish the severity of disease of previously treated and new DR-TB patients.

3.5 Hypothesis

Scientific Hypothesis: It is anticipated that in patients receiving DR-TB treatment containing Bedaquiline, previously treated TB patients will require a longer duration of treatment to achieve culture conversion as compared to new DR-TB patients.

Primary Statistical Hypothesis

H_A: Previous exposure to TB treatment will be associated with increased time to culture conversion in patients receiving DR-TB regimen containing Bedaquiline as compared to newly diagnosed DR-TB patients.

$H_{O:}$ Previous exposure to TB treatment will not be associated with increases time to culture conversion in patients receiving DR-TB regimen containing Bedaquiline as compared to newly diagnosed DR-TB patients.

Secondary Statistical Hypothesis

Objective 1

$H_{A:}$ Time to culture conversion will differ depending on treatment regimen

Ho: Time to culture conversion will not differ depending on treatment regimen

Objective 2

H_A: The rate of relapse will be greater in the previously treated as compared to new DR-TB patients
H_O: The rate of relapse will be the same in the previously treated and new DR-TB patients
Objective 3
H_A: The duration of Bedaquiline therapy will be longer in the previously treated as compared to new DR-TB patients
H_O: The duration of Bedaquiline therapy will be the same in the previously treated and new DR-TB patients

Objective 4
H_A: DR-TB patients with previous exposure to TB treatment will have greater severity of disease than new patients
H_O: DR-TB patients with previous exposure to TB treatment will have the same severity of disease as new patients

4 Methods

4.1 Study Design

A retrospective cohort study will be undertaken for confirmed DR-TB patients who were initiated on DR-TB regimen containing Bedaquiline from April 2016 to March 2019 at Sizwe Tropical Disease Hospital. The choice of study design is appropriate as strict eligibility criteria are maintained for consideration of Bedaquiline therapy, thus a retrospective cohort study will ensure an adequate sample size will be reached. The time period will allow for assessment of culture conversion status for majority of the patients.

This study will account for changes to the DR-TB programme clinical guideline in South Africa from 2016 to 2019. It is anticipated that patients diagnosed with DR-TB during this period would have been initiated on a combination of Bedaquiline and Ethionamide or Bedaquiline and Linezolid and a suitable background regimen. Diagnosis of drug resistant TB is based on GeneXpert MTB Rif assay/ GeneXpert Ultra assay (since November 2017), Culture, Drug Susceptibility Testing or Line Probe Assay testing results. Drug susceptibility testing is performed by the National Health Laboratory Services for the following drugs: Rifampicin; Isoniazid; Second line injectables; and fluoroquinolones. All patients initiated on the Bedaquiline containing regimen are monitored for ECG changes and adverse drug reactions. Monitoring of the QT interval on ECG, with prolongation of the corrected QT defined as a value >450 ms in males or > 470ms in females or > 60ms increase from baseline would be considered as significant and warrants interruption or stopping of Bedaquiline therapy.

Sputum culture conversion is considered as one negative culture from a positive baseline culture result as per clinical protocol at Sizwe Tropical Disease Hospital. The date of the sputum collection of the negative culture will be regarded as the date of culture conversion. Culture conversion will be used as a surrogate end point for treatment outcome to account for patients who are still currently on treatment or have been discharged from Sizwe Tropical Disease Hospital to ambulatory care at local treatment facilities.

Setting and Study Population

The study will be conducted at Sizwe Tropical Disease Hospital in Gauteng Province. Sizwe Tropical Disease Hospital serves as a referral centre for complicated MDR/XDR-TB cases in Gauteng. Patients are initiated on treatment in-hospital and discharged on culture conversion for follow-up at local health facility. The study population will consist

of all patients receiving the DR-TB treatment containing Bedaquiline with documented culture conversion

4.2 Subjects

Inclusion Criteria

Bacteriologically confirmed DR-TB

All patients receiving DR-TB Regimens containing Bedaquiline including new DR-TB patients with no previous history of TB treatment

Documented culture conversion

Exclusion Criteria

No culture results at start of treatment

Negative culture results at start of treatment

Extrapulmonary DR-TB

Mixed Infection (i.e. Drug Sensitive and Drug Resistant TB or Non Tuberculous Mycobacteria (NTM) and Drug Resistant TB)

GeneXpert MTB Rif assay negative at start of treatment

4.3 Sampling Technique

All patients initiated on DR-TB regimen containing Bedaquiline from April 2016 will be identified for review. The statistics for Sizwe Tropical Disease Hospital for the period April 2016 to March 2019 showed that a total of 1174 DR-TB patients were initiated on TB regimen containing Bedaquiline. Of the DR-TB patients, 951 were treated for MDR-TB, 151 were treated for pre-XDR TB and 72 were treated for XDR-TB. From incidence estimates in the literature, it is anticipated that the ratio for new DR-TB patients to previously treated DR-TB patients will be 1:4. [25]

4.4 Study Procedure

Suitable patient profiles meeting the inclusion criteria will be identified from the TB Registers. A list of patient file numbers will then be finalised for file will be retrieved by the hospital clerks. This method will ensure that the patient privacy and confidentiality will be maintained. Anonymised patient data will then be extracted from files. Patient's demographic information, category of TB patient, information on severity of TB disease, treatment regimens and treatment response parameters (sputum/culture conversion, adverse events, and treatment outcomes) will be extracted. The patient information will be captured on Case Report Forms (CRF). CRFs will be filed in secure storage area.

4.5 Measures

Explanatory Variables

DR-TB Case Definitions:[26]

MDR-TB case is defined as "a patient with bacteriologically proven TB with resistance to Rifampicin and Isoniazid with or without resistance to other first-line anti-TB drugs".

Pre XDR-TB case is defined as "MDR-TB with additional resistance to either a second-line injectable or a fluoroquinolone".

XDR-TB case is defined as “MDR-TB that also has resistance to at least a fluoroquinolone and one second-line injectable (Amikacin, Kanamycin and/or Capreomycin)”.

Patients will be categorised as follows:[26]

Category I: New	“A patient who has received no anti-tuberculosis treatment for TB, MDR- or XDR-TB or received less than one month of anti-tuberculosis drugs”
Category II: Previously treated with first-line drugs only	“Patient who has been treated for one month or more for TB with only first-line drugs”.
Category III: Previously treated with second-line drugs	“Patient who has been treated for one month or more for TB or DR-TB with one or more second-line, with or without first-line drugs”.

Severity of disease:

Severity of disease will be classified according to sputum smear grading, baseline CXR findings and baseline resistance patterns.[27]

Sputum smear will be graded on ZN Stain as follows:

“Negative: No acid-fast bacilli (AFB) observed”

“Scanty: 1-9 AFB in 100 fields”

“1+:10-99 AFB in 100 fields”

“2+:1–10 AFB in 1 field”

“3+: > 10 AFB in 1 field “

CXR findings will be categorised as

cavitatory –single

cavitatory - multiple

non-cavitatory.

Baseline resistance patterns will be assessed (if documented in laboratory report) as presence of:[26]

Resistance to Isoniazid: inhA gene mutation

KatG gene mutation

Resistance to Rifampicin: RpoB gene mutation

Resistance to second line injectables: rrs gene mutation

eis gene mutation

Resistance to Fluoroquinolones: gyra A

gyra B

Treatment regimen assessed will be reflective of current and previous South African National TB guidelines.[26]

Standardised DR-TB Bedaquiline containing Regimens:

1. Short Regimen:

Duration: 9-11 months

Duration of Intensive Phase: 4 months

Duration of Extended Intensive Phase: 6 months

Current Standard Drug Regimen: Linezolid (LZD) (first two months) +Bedaquiline (BDQ) + Levofloxacin (LFX)+ Clofazimine (CFZ)+ Isoniazid (INH) (high dose) + Pyrazinamide (Z) + Ethambutol (E)

Previous Standard Drug Regimen:

Ethionamide (Eto) + BDQ+LFX+ CFZ + IINH (high dose) + Z +E

2. Long Regimen:

Duration: 18-20 months

Duration of Intensive Phase: 6 months

Duration of Extended Intensive Phase: 8 months

Current Standard Drug Regimen: LZD + BDQ+LFX+CFZ+Terizadone (TRD)

Previous Standard Drug Regimen:

Eto+BDQ+LFX+CFZ+TRD

Other factors (confounders) that may contribute to time to culture conversion will be extracted from patient records:

Sociodemographic information such as Gender, Age, Weight, smoking history will be collected from patient records.

History of other medical conditions such as Diabetes Mellitus, Hypertension, Renal Disease, Hearing Loss will be collected from patient records.

HIV status information and relevant information pertaining to Anti- Retroviral therapy start date , Anti -retroviral Therapy regimen, any modifications to Anti -retroviral regimen and last viral load will be collected from patient records where applicable.

Outcome Variables:

Clinical endpoints will be considered as follows:

Sputum culture conversion is defined as one consecutive negative culture as per clinical protocols for Sizwe Tropical Medicine Hospital and will reflect the date of sputum collection.

Time to culture conversion will be the date from treatment initiation to date of culture conversion.

Duration of Bedaquiline treatment will be the date of Bedaquiline initiation to date of stopping Bedaquiline treatment.

Specific Measurements:

DR-TB Treatment start date: Documented date of treatment initiation.

Evidence of relapse: Positive culture results following documented culture conversion.

Culture conversion date: Documented date of negative culture result with subsequent negative culture outcomes.

Time to culture conversion (days): Calculated period from date of treatment initiation to date of culture conversion.

Bedaquiline start date: Documented date of Bedaquiline initiation.

Bedaquiline end date: Documented date of stopping Bedaquiline.

Duration of Bedaquiline treatment (days): Calculated period from date of initiation to stopping Bedaquiline.

Specific information to be extracted from patient records for each objective has been outlined below:

Primary Objective: To compare the time to culture conversion for previously treated and new DR-TB patients receiving the Bedaquiline containing regimen

DR-TB patients receiving the Bedaquiline containing regimen will be categorised as new or previously treated TB patients according to DR-TB category definitions. Data on DR-TB treatment start date and culture conversion date will be extracted from patient records and the time to culture conversion (in days) will be calculated from the data.

Secondary Objectives:

1. To evaluate treatment regimens (Linezolid or ethionamide containing regimens) at time of culture conversion for previously treated and new DR-TB patients

DR-TB patients receiving the Bedaquiline containing regimen will be categorised as new or previously treated according to DR-TB category definitions. Information on the individual drugs used in the treatment regimen at the time of culture conversion will be extracted for each patient. Additional information on the drug dosage of Linezolid at the time of culture conversion will also be extracted if applicable.

2. To compare the rate of relapse in previously treated and new DR-TB patients receiving the Bedaquiline containing regimen

DR-TB patients receiving the Bedaquiline containing regimen will be categorised as new and previously treated according to DR-TB category definitions. Information pertaining to monthly

culture results and culture conversion date will be reviewed from patient records. Relapse will be determined by an initial culture conversion documented in the patient record followed by positive monthly culture result.

3. To compare the duration of Bedaquiline therapy in previously treated and new DR-TB patients

DR-TB patients receiving the Bedaquiline containing regimen will be categorised as new or previously treated TB patients according to DR-TB category definitions. Information on the start date of Bedaquiline and the end date of Bedaquiline treatment will be extracted from patient records and the duration of Bedaquiline treatment (in days) will be calculated from the extracted data.

4. To establish the severity of disease of previously treated and new DR-TB patients

DR-TB patients receiving the Bedaquiline containing regimen will be categorised as new or previously treated TB patients according to DR-TB category definitions. Information on baseline smear grading, baseline CXR findings and baseline resistance testing will be extracted from patient records in order to establish the severity of the disease.

5 Data Management Plan

5.1 Data Collection

Patient information will be captured on paper-based Case Report Forms (CRF) (see Appendix1). To ensure that the data will be anonymised, patient identification numbers will be assigned to each of the audited files. The CRF will consist of specified data categories to assist with capturing of information, from diagnosis of DR-TB to treatment outcome.

5.2 Data entering, storage and validation

The information captured on the CRF will be checked for completeness. Any errors or omissions will be corrected immediately. The CRFs will be cross-referenced with the source documents to validate entries by the researcher. Study files will be labelled and kept in a secure, locked location with limited access. The anonymised data will be transferred onto an excel spreadsheet for further analysis using STATA version 15.0 software (StataCorp, Texas 2019) on password protected laptop. An external hard drive will be used to backup data.

6 Statistical Considerations

Sample Size and Power

Using WinPepi Version 11.65 software, a comparison of two independent groups, the group sample sizes were determined to be 76 (exposed) and 304 (unexposed) to achieve power of 80% to detect a moderate difference in comparison between the two groups, with a level of significance of α 0.05. These computations were done based on the median survival times derived from a previous study.[25] The study findings showed that culture conversion rates were 30.1% at 30 days, 56.7% at 60 days, 80.5% at 90 days and 91.2% at end of treatment.[25] The median survival time of 180 days was based on the worst-case scenario from findings from the Borisov et al study.[25] The range of values are detailed below:

Difference between groups	HR	Median survival times (days)		Total Sample	Required Sample	
		Exposed (A)	Unexposed (B)		Exposed (A)	Unexposed (B)
Large	0.333	180	60	145	29	116

Moderate	0.556	180	100	380	76	304
Small	0.714	140	100	870	174	696

Patient records will be systematically randomly sampled (every 2nd patient record) until the required sample size for the two groups are achieved.

Data Analysis Plan

Descriptive Analysis

Patient characteristics will be summarised using frequencies and percentages for categorical variables and median and interquartile ranges (IQRs) for continuous variables.

Unadjusted Analysis

Odds ratios and Chi-squared tests will be used to determine associations between category of patient and conversion. Time to initial conversion of sputum culture will be analysed using Kaplan-Meier survival curves, which will be plotted, and the log rank test will be used to determine differences between groups. A p value ≤ 0.05 will be considered as statistically significant.

Adjusted Analysis

We will use Cox proportional hazards modelling to estimate hazard ratios, with time to sputum culture conversion as the endpoint, while controlling for confounding variables such as HIV status, ART, comorbidities.

7 Ethical Considerations

This protocol will be submitted to the Human Research Ethics Committee of Stellenbosch University for ethical approval. The protocol and a letter of request for permission to conduct the study will submitted to the CEO and management of Sizwe Tropical Disease Hospital.

Informed Consent

Retrospective patient record review, informed consent will not be possible from patients. Institutional permission to conduct the study will be requested to ensure access to patient records. An application for waive of consent will be applied for from the HREC, Stellenbosch University. The research involves use of routinely collected data and no participant contact will be sought. The waiver will not adversely affect the rights and welfare of the participants.

Confidentiality

All personal identifiers will be coded; all patient records will be assigned a personal identification number to protect data privacy and confidentiality.

Standard of Care

This is a retrospective file review; all patient identifiers will be anonymised. The data will be anonymised at the point of data collection no identifying information such as patient file numbers, names, or contact details will be recorded in any way during data collection. Once data has been collected, there is no way of tracing it back to the original patient/participant. Only aggregated data will be presented and anonymised in the reporting of findings i.e. no individual cases will be reported on.

Inclusion of Women

This study is a retrospective file review, all MDR-TB patients receiving the DR-TB

Regimen containing Bedaquiline will be included in the study.

Exclusion of Children

This study is a retrospective file review, all MDR-TB patients receiving the DR-TB

Regimen containing Bedaquiline will be included in the study. Consent for inclusion will not be sought in the case of minors as this study is a retrospective review of patient records.

Conflict of Interest

No conflict of interests is anticipated.

Dissemination of Findings

The results of this study will be disseminated to Sizwe Tropical Disease Hospital, Dr N Ndjeka from the Department of Health, University of Stellenbosch, my peers (Conferences-Stellenbosch University; The Union and Journal Publication).

8 Study Timelines

Activity/Month	Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
Protocol Development	X	X																
Letters of Permission		X	X															
Ethics Application				X	X	X	X	X	X	X	X							
Data collection upon ethics approval												X	X	X	X			
Data analysis																X		
Write-up																	X	
Submission for examination																		X

9 Study Limitations

Methodological limitation is that we will be using a surrogate marker for treatment response and not evaluating treatment outcomes.

This study will provide information on clinically ill patients who would require in-patient care; thus, it would be difficult to generalise our findings to Primary Health Facilities.

Due to retrospective file review, missing information may affect the quality of data. All source documents related to clinical monitoring contained in the patient file will be reviewed to ensure a complete data.

10 Feasibility

This study will be undertaken by reviewing patient records for a fixed duration. The proposed period for file review is adequate to obtain an adequate sample. The sample size of 380 required for the study is achievable. Since Sizwe Tropical Medicine Hospital is a Centre of Excellence, data on the severity of the disease, resistance profiles and co-morbidities will be available.

11 Future Directions

The findings of this study will provide added information on the programmatic implementation of the drug resistant policy framework regarding Bedaquiline containing regimens.

12 Study Team

The Principal Investigator will be responsible for data collection. The study supervisors will provide statistical and technical support. The hospital staff will assist with file retrieval.

13 Institutional Research Environment

The Clinical Epidemiology Department, Stellenbosch University, Western Cape, South Africa headed by Professor T Young provides academic support to both Masters and PhD students and is extensively involved and well experienced in conducting research. The division has a multi-disciplinary team with Biostatistical support provided by a team of statisticians. The division has access to internet, emails, photocopying facilities, telephone and fax services. The Clinical Epidemiology Department is well supported by Research Capacity Development Funding (RFO), ensuring capacity development of staff and post graduate students. The RFO provides support for annual research outputs reporting.

14 Budget

Period: January 2020 to August 2020

Item	**Description**	**Units**	**Unit Cost (VAT incl)**	**Total Cost (VAT incl)**
Consumables				
Printing	Case Report forms for data collection	380	(0.45c/page x 4 pages) = R1.80	R 684
Office supplies	A4 files and stationery	1	R1 000	R1 000
Research Travel				
Conference Costs	Conference attendance on acceptance of abstract	1	R20 000	R20 000
Travel	Fuel costs	40	(3.61/km x94km return) = R340	R13 600
Other direct costs				
	Internet, Email	3	250	R750
	Cell Phone	20	(0.79/min x60min) = R47.40	R948
	Equipment (Ext Hard Drive 2TB)	1	1500	R1 500
Publication	Open Access Journal	1	R12 000	R12 000

Total				R50 482

Budget Motivation:

Equipment will be purchased to ensure that study data is safely and securely backed up.

Travel costs to the study facility comprises fuel costs to cover 94km return trip per visit during the data collection phase of the study.

Office supplies will be purchased to ensure that the paper based CRFs are securely filed and stored.

Internet data is required during the data collection period and analysis for communication with statistician.

Cell phone charges are based on average one-hour conference calls to update supervisors on study progress every two weeks upon ethics approval.

An application for a Travel Grant will be made to Stellenbosch University to cover conference costs to attend The Union TB Conference in 2020.

An application will be submitted to The Library at Stellenbosch University to assist with publication costs.

The application for funding to The Harry Crossley Fund to cover operational costs of the study has been successful.

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CRF 001 Previous tuberculosis treatment history and efficacy of bedaquiline

Date: PID no:

1. Gender

Male =1
Female=2

2. DOB D D M M Y Y Y Y

3. Age

4. Weight . KG

5. Smoker

No=0
Yes=1

6. Comorbidity

No=0
Yes=1

6a. If Yes to Q6.

DM=1
HPT=2
Renal Disease=3
Hearing Loss=4
Other

If Other, specify:

7. HIV STATUS

Negative=0
Positive=1
Unknown=8

8. ART started

No=0
Yes=1

8a. ART Start Date D D M M Y Y Y Y

8b. Modification of ART regimen

No=0
Yes=1

8c. ART Regimen *Tick Appropriate drugs*

TDF
FTC/3TC
ABC
EFV

NVP
LPV/r or ATV/r
DTG

8d. Last Viral Load
<400=0
>400=1

9. Date of DR-TB diagnosis D D M M Y Y Y Y

10. Laboratory diagnosis

Culture + DST=0
GXP=1
LPA=2
All tests=3
Cul+DST+LPA=4
GXP+Cul+DST=5
GXP+LPA=6

12. DR-TB Treatment Start Date D D M M Y Y Y Y

13. Category of patient

Category I=0
Category II=1
Category III=2

14. Baseline Smear Grading

Negative=0
Scanty=1
1+=2
2+=3
3+=4

15. Baseline CXR findings

No Cavitation=0
Cavitation (single)=1
Cavitation (multi)=2

16. Baseline Resistance testing, detection of the following mutation/s **Tick appropriate box**

inhA	☐
KatG	☐
RpoB	☐
SLID mutation	☐
gyraA	☐
gyraB	☐

17. Culture Conversion Date D D M M Y Y Y Y

18. Time to Culture Conversion ☐☐☐☐ DAYS

19. Bedaquiline start date D D M M Y Y Y Y

20. Bedaquiline end date D D M M Y Y Y Y

21. Duration of Bedaquiline ☐☐☐☐ DAYS

22. Treatment Completion Date D D M M Y Y Y Y

23. Treatment Outcome ☐

Cured=1

Tx Completed=2

LTFU=3

Tx Failure=4

Died=5

Still on Rx=6

Not Evaluated=7

Transferred out=8

24. Treatment Regimen ***Tick all appropriate drugs***

Amikacin		Imipenam/cilas	
Amox/Clav		Isoniazid	
Bedaquiline		Linezolid	
Clofazimine		Meropenem	
Cycloserine		PAS	
Delaminid		Pyrazinamide	
Other, please specify			

If Linezolid included in regimen, go to Q24a

24.a Linezolid dosage

300mg=0

600mg=1

1200mg=2

25. Adverse Events

No=0

Yes=1

If Yes, specify

26. Change of Regimen

No=0

Yes=1

If Yes, go to Q26.a

26a. Was BDQ stopped

No=0

Yes=1

If Yes, please specify reason

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Approval Notice

New Application

22/05/2020

Project ID: 11609

HREC Reference No: S19/09/177

Project Title: Previous tuberculosis treatment and efficacy of Bedaquiline

Dear Dr Amashnee Saimen

The **Response to Modifications** received on 03/04/2020 16:48 was reviewed by members of **Health Research Ethics Committee** via **expedited** review procedures on 22/05/2020.

Thank you for attending to the requested modifications, the research protocol is now finally approved. Please note the following information about your approved research protocol:

Protocol Approval Date: 22 May 2020

Protocol Expiry Date: 21 May 2021

Please remember to use your Project ID 11609 and Ethics Reference Number S19/09/177 on any documents or correspondence with the HREC concerning your research protocol.

Please note that the HREC has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

After Ethical Review

Translation of the informed consent document(s) to the language(s) applicable to your study participants should now be submitted to the HREC.

Please note you can submit your progress report through the online ethics application process, available at: Links Application Form Direct Link and the application should be submitted to the HREC before the year has expired. Please see *Forms and Instructions* on our HREC website (www.sun.ac.za/healthresearchethics) for guidance on how to submit a progress report.

The HREC will then consider the continuation of the project for a further year (if necessary). Annually a number of projects may be selected randomly for an external audit.

Provincial and City of Cape Town Approval

Please note that for research at a primary or secondary healthcare facility, permission must still be obtained from the relevant authorities (Western Cape Departement of Health and/or City Health) to conduct the research as stated in the protocol. Please consult the Western Cape Government website for access tothe online Health Research Approval Process, see: https://www.westerncape.gov.za/general-publication/health-research-approval-process. Research that will be conducted at any tertiary academic institution requires approval from the relevant hospital manager. Ethics approval is required BEFORE approval can be obtained from these healthauthorities.

We wish you the best as you conduct your research.

For standard HREC forms and instructions, please visit: Forms and Instructions on our HREC website https://applyethics.sun.ac.za/ProjectView/Index/11609

If you have any questions or need further assistance, please contact the HREC office at 021 938 9677.

Yours sincerely,

Mrs. Brightness Nxumalo
HREC 2 Coordinator

National Health Research Ethics Council (NHREC) Registration

Number: REC-130408-012 (HREC1) •REC-230208-

010 (HREC2)

Federal Wide Assurance Number: 00001372

GAUTENG PROVINCE
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REPUBLIC OF SOUTH AFRI CA

Enquiries: Dr. M.C. Louw Tel: 011 531-4305
Fax: 011 531-4377
Date: 10 September 2019

Dr Amashnee Saimen

RE: DOES PREVIOUS TUBERCULOSIS TREATMENT HISTORY INFLUENCE TIME TO CULTURE CONVERSION FOR PATIENTS RECEIVING A BEDAQUILINE CONTAINING REGIMEN AT SIZWE TROPICAL DISEASE HOSPITAL, SOUTH AFRICA?

Sizwe Tropical Disease Hospital supports the study titled: "Does previous tuberculosis treatment history influence time to culture conversion for patients receiving a Bedaquiline containing regimen at Sizwe Tropical Disease Hospital, South Africa?", to be conducted in our facility.

Kind regards

Dr. M.C. Louw
CEO: Sizwe Tropical Disease Hospital

;iAUTENGHEALTH DEPARTMENT I
Sizwe Tropical Disease Hospit al
Private Bag X2
Sandringham **2131**
DR. M. C. LOUW
Chief Executive Officer
Date: 10/10/2019

STELLENBOSCH UNIVERSITY
FACULTY OF MEDICINE AND HEALTH SCIENCES

TO WHOM IT MAY CONCERN

ASSIGNMENT/THESIS/DISSERTATION RELEASE

Student's surname	Saimen		
Initials	A	Student no	
Title of assignment/thesis/dissertation: Influence of previous tuberculosis treatment history on time to culture conversion for patients receiving a Bedaquiline containing regimen at Sizwe Tropical Disease Hospital, South Africa			
Faculty	Department of Global Health		
Division/Department	Division of Epidemiology and Biostatistics		
Degree	MClinEpi		
Supervisor (s)	Tonya Esterhuizen, co-supervisor: Xavier Padanilam		

I confirm that

- I and the co-supervisor(s) (if applicable) have read the final draft of the assignment/thesis/dissertation
- The assignment/thesis/dissertation is ready for examination
- The assignment/thesis/dissertation has been checked using anti-plagiarism software

Supervisor signature: **Date: 26.02.2021**

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